

OXYTETRACYCLINE TAGS IN PINK SALMON FRY APPLIED BY  
IMMERSION AND DETECTED BY FLUORESCENCE SPECTROMETRY

By

Timothy Kent Page

RECOMMENDED:

Robert Jagen  
Member

Jerry J. Warner  
Member

Michael R. Stoltz  
Advisory Committee Chair

William D. Stoltz  
Department Head

APPROVED:

Alan  
Dean, School of Fisheries and Ocean Sciences

[Signature]  
Dean of Graduate School

4/27/89  
Date

OXYTETRACYCLINE TAGS IN PINK SALMON FRY APPLIED  
BY IMMERSION AND DETECTED BY FLUORESCENCE SPECTROMETRY

A  
THESIS

Presented to the Faculty of the  
University of Alaska Fairbanks

In Partial Fulfillment of the Requirements  
For the Degree of  
MASTER OF SCIENCE

By  
Timothy Kent Page, B.S.  
Juneau, Alaska  
May 1989

BIOSCI  
SH  
156.8  
P34  
1989

BIOSCIENCES LIBRARY  
UNIVERSITY OF ALASKA FAIRBANKS



## ABSTRACT

I investigated the feasibility of using spectrofluorescence as a quantitative method of analyzing pink salmon fry otoliths for the presence of oxytetracycline (antibiotic). I exposed twenty-four groups of pink salmon fry (approximately 250 individuals each) to solutions of oxytetracycline ranging in concentration from unexposed to 2000 ppm, and in duration of exposure from one to twelve hours. Otoliths from unexposed fry had the lowest mean fluorescence ( $\log e$  [mean flu. units] = -1.77). Otoliths from fry exposed to 500 ppm for twelve hours had the highest fluorescence ( $\log e$  [mean flu. units] = 0.899).

Fluorescence increased nonlinearly with duration of exposure. There is a linear increase of fluorescence with exposure up to 500 ppm; above which fluorescence decreases. Pink salmon fry exposed to OTC in solution absorb detectable amounts of OTC in their otoliths. These amounts of OTC can be quantitatively measured by spectrofluorometry.

## TABLE OF CONTENTS

	Page
ABSTRACT . . . . .	3
LIST OF FIGURES. . . . .	5
LIST OF TABLES . . . . .	6
ACKNOWLEDGEMENTS . . . . .	7
I. INTRODUCTION. . . . .	8
II. MATERIALS AND METHODS . . . . .	15
A. Exposure to OTC . . . . .	15
B. Spectrofluorometric Analysis. . . . .	18
C. Statistical Analysis. . . . .	22
III. RESULTS . . . . .	23
A. Response to Treatment . . . . .	23
B. OTC Uptake vs. Duration of Exposure . . . . .	26
C. OTC Uptake as a Function of Immersion Concentration . . . . .	33
IV. DISCUSSION . . . . .	42
A. Support of Previous Works . . . . .	42
B. Marking Cost. . . . .	53
C. Tagging Adults. . . . .	53
V. CONCLUSIONS. . . . .	55
VI. LITERATURE CITED . . . . .	56

# LIST OF FIGURES

	Page
Figure 1. Standard curve of oxytetracycline dissolved in 5% TCA (w/v). Total volume of sample equal 1.0 ml . . . . .	27
Figure 2. Scatterplots of paired otolith fluorescence vs. duration of exposure within the same concentration. . . . .	28
Figure 3. Mean amounts of OTC present in each otolith pair for the different exposures within the same concentration . . . . .	30
Figure 4. Notched box plots of paired otolith fluorescence vs. duration of exposure in the same concentration. . . . .	31
Figure 5. Scatterplots of paired otolith fluorescence vs. concentration within the same duration of exposure. . . . .	35
Figure 6. Mean amounts of OTC present in each otolith pair for the different concentrations within the same duration of exposure . . . . .	37
Figure 7. Notched box plot of paired otolith fluorescence vs. OTC concentration within the same duration of exposure . . . . .	38
Figure 8. Schematic diagram of uptake and deposition of OTC from the ambient water into the fry's otoliths and other body parts. . . . .	48

## LIST OF TABLES

	Page
Table 1. Water chemistry of reservoirs. Record of dissolved oxygen, pH and temperature. . . .	24
Table 2. Regression summaries of the concentration groups . . . . .	34
Table 3. Regression summaries of the durations of exposure . . . . .	41
Table 4. Estimated amounts of OTC present in fry otoliths . . . . .	44

## ACKNOWLEDGEMENTS

I am grateful to the Alaska Department of Fish and Game Fisheries Rehabilitation and Enhancement Division for funding, in particular to Karen Crandall, Bill Johnson and Sam Bertoni for their assistance and use of the computer system. I am also grateful to Douglas Island Pink and Chum for funding and facilities, in particular, to Ladd Macaulay, Mark Hundley, and Steve Reid. I am indebted to Dr. Michael Stekoll for help in developing the chemical assay, Michael Rowcroft for assistance and encouragement, and Herman Savikko for proofreading. I thank my committee for their time spent reviewing this manuscript.

## INTRODUCTION

The problems of salmon fishery management in Southeast Alaska are intensified by the difficulty of managing individual stocks which are harvested in mixed stock fisheries. To manage a fishery effectively, hatchery and wild stocks must be separately identifiable (Weber and Ridgway 1967). Accurate stock separation is important to all salmon fishery management, but it is particularly important in Alaska to be able to estimate hatchery contributions to a fishery. Hatchery contributions to mixed stock fisheries are significant in Alaska. These mixed stock fisheries must be managed effectively to prevent over-fishing of the wild stocks and to ensure that hatcheries have sufficient fish returns for brood stock and cost recovery (Stekoll et al. 1986).

Tagging of hatchery fish has been one historic method of distinguishing fish in a mixed stock fishery. Several methods of tagging salmon have been used. These tags include fin clipping, stock specific parasites, genetic markers and coded micro wire (Scidmore and Olsen 1969; Gharrett et al. 1983 and Jefferts et al. 1963). Although all of these methods of tagging salmon are effective in specific instances, they each have disadvantages when applied en masse to hatchery fish.



Fin clipping is an effective tagging method when applied to small numbers of fish. However, when applied to a larger number of fish it becomes time consuming, mutilates fish and involves the handling of individual fish which can introduce disease (Scidmore and Olsen 1969). These factors make fin clipping impractical and potentially harmful for mass-tagging hatchery fish.

Stock specific parasites work well for the identification of an affected stock. This method of stock separation has been used to separate sockeye salmon from Bristol Bay and sockeye from Kamchatka (Margolis 1963). The cestoda Triaenophorus crassus is found only in the sockeye stocks of Bristol Bay and the nematode Dacnitis truttae is found only in sockeye stocks of Kamchatka. This phenomenon allows the two stocks to be separated in a mix stock fishery. However, the likelihood of a particular hatchery stock having a unique parasite is rare.

Genetic marking is a recently developed method of marking a stock of fish (Gharrett et al. 1983). Although this method of stock identification has been proven to work, it does take genetic expertise and research time to develop (Gharrett et al. 1983). Genetic marking methods are designed to discriminate among stocks, and require planning which includes: 1) determination of genetic composition of marked and unmarked stocks, 2) the level of



marking effort possible, and 3) the desired level of discrimination. These factors make stock separation through genetic marking beyond the scope of most hatchery managers.

The stock tagging method most widely used in Alaska is the coded wire tag (Jefferts et al. 1963). This method involves the injection of a small micro-wire between the olfactory sacs of emergent or presmolt salmon. These wires are marked with a binary code which can identify the fish's origin and other information about the fish. This method of tagging salmon has proven effective in the separation and estimation of stock contributions in mixed stock fisheries when significant numbers of tags can be recovered from harvested fish. However, coded wire tagging usually tags a relatively small portion of the total fish released. This fact makes it imperative that there is an intensive system of recovery of the tagged fish in the terminal fishery.

The 1988 planned coded wire tagging for all salmon species and steelhead trout in Southeast and Southcentral Alaska is 4,060,000 (Crandall 1988). The major disadvantage of this large tagging effort is the expense of recovering the tagged fish from the various fishery ports and the subsequent decoding of those tags. Initially salmon fry must have their adipose fins removed and be

injected with tags by a crew averaging four people. Included in this stage is the cost of the tags, labor, and machinery to inject the tags. In the terminal fishery there is the cost of port sampling to recover the tags. Finally there is the cost of removing and reading the tags in the laboratory. The cost from injection to recovery and extraction averages \$200.00 per tag recovered (Sam Bertoni, Alaska Department of Fish and Game, personal communication).

An alternative solution to this stock separation problem is to apply an identifying tag to all the fry of a given stock. One potential tag might be a calcium-binding chemical such as the antibiotic oxytetracycline (OTC). Tetracycline antibiotics have proven to be effective in the tagging of fish, (Brothers 1985, Tsukamoto 1985, Koenings et al. 1983, Odense and Logan 1974, Weber and Ridgway 1967, Kobayashi et al. 1964, Ibsen et al. 1963, Kohn 1961 and Weber 1962), and the antibiotics are FDA approved for fisheries use (Schnick and Meyer 1979).

The methods most frequently used to administer tetracycline antibiotics are by immersion or by feeding. Either of these methods results in the OTC being absorbed into the fish's body where it binds with calcium in the bones of the fish (Ibsen and Urist 1969). The OTC is absorbed through the skin and gills and laid down in the

calcareous tissues of the fish such as the skeletal bones, dermal bones and otoliths. After a fish has been exposed to OTC it carries with it a chemical tag which might be used to separate it from other stocks of fish.

The tetracycline family of drugs, OTC included, have ultraviolet fluorescent properties. These properties make it possible to detect OTC in bone tissues by ultraviolet microscopic inspection and chemical extraction analyses (Weber and Ridgway 1967; Koenings and Lipton 1983). Fluorescence results when a molecule absorbs light energy which raises the molecule to an excited state. Upon return to its ground state the molecule emits light energy (fluorescence) (Weber and Ridgway 1967). Mass-tagging fish with OTC is quick, inexpensive and easy to administer whether applied as a dietary component or in immersions.

In the past, researchers who tagged fish with OTC analyzed the bony structures visually by fluorescence microscopy (Hettler 1984). Detection of the chemical tag using this method depends on the subjective observation of the researcher as he/she views the ultraviolet illuminated bone tissue through a microscope (Weber and Ridgway 1962; Scidmore and Olsen 1969 and Kobayashi et al. 1964). Samples analyzed this way can only be qualitatively categorized.

Fluorescence photofluorometry on the other hand, can detect OTC which has been chemically extracted from the otolith and is quantitatively accurate at concentrations above 0.01  $\mu\text{g/ml}$  (Stekoll et al. 1986). If the OTC present in an otolith can be extracted in more concentrated solutions than 0.01  $\mu\text{g/ml}$  then it would be a useful tag.

I set out to chemically tag pink salmon fry in a hatchery setting. This would give an indication of the practicality of applying OTC as a tag to hatchery stocks. I exposed fry to the OTC in baths. I chose this method over other methods such as administering OTC in feed because immersion is a direct method of application. Immersing insures that each fry has the same exposure to OTC rather than depending on them ingesting the OTC in the feed. Exposing the fry to OTC in solution also permits marking earlier in embryonic development. I exposed the fry to different concentrations and durations of OTC to determine optimum conditions of tagging.

I used spectrofluorometric methods to detect the OTC in the otoliths of the fry. I analyzed solutions of otoliths with a spectrophotofluorometer and determined the optimal tagging treatment. I chose the otolith as the structure to be analyzed because it is calcified early in the embryonic development of pink salmon fry. Also, the calcified components of the otolith are less susceptible to

breakdown during metabolic processes than are other bony structure of the fish's body (Neilson et al. 1985).

The hypotheses are as follows:

1. Pink salmon fry can be measurably tagged in the otolith by immersion in solutions of OTC.
2. OTC laid down in the otoliths of pink salmon fry can be released into solution by dissolving the otolith in TCA and detecting it by fluorescence photofluorometry.
3. The fluorescence of extracted OTC from an exposed otolith increases as concentration and time of exposure increase.



## MATERIALS AND METHODS

## EXPOSURE TO OTC

I carried out the marking portion of this experiment in spring 1986 at the Douglas Island Pink and Chum (DIPAC) Sheep Creek Hatchery, Juneau, Alaska. At exposure time the fry had closed ventral sutures and had incubated for approximately seven months. All of the fry were in excellent health.

Three days before treatment I collected twenty-four groups numbering 250 pink salmon fry each from a Zinger hatchery incubator (NOPAD S40LZ). I siphoned the fry from the incubator into a bucket and then poured them into a Heath tray. I placed the twenty-four exposure groups into six separate Heath incubation cabinets. I supplied these cabinets with six concentrations of OTC (control or 0, 100, 250, 500, 1000, and 2000 parts per million (ppm, mg/l)). Within each of these six cabinets I placed four trays, which were exposed to OTC solutions for 1, 3, 6 and 12 hours. I placed each cabinet on a separate 180 liter fiberglass reservoir. Submersible pumps in the reservoirs pumped a total of six gallons per minute through each of the cabinets. I provided each of the incubator trays with artificial substrate (BioRings). Before and after exposure I provided the cabinets with a continuous supply of

hatchery water.

I estimated the total volume of each of the Heath stack systems to be 184 liters. To begin the exposure I temporarily shut off the water flow, poured 136 liters of water into each of the reservoir tanks and weighed the amounts of OTC needed to bring the six reservoirs to the desired concentrations of 0, 100, 250, 500, 1000 and 2000 ppm. I then dissolved these amounts of OTC in buckets, buffered the solutions to pH 7.0 with sodium bicarbonate, poured the solutions into the respective reservoirs and stirred them thoroughly.

I started the pumps to begin circulation and exposure of the fry to their respective solutions of OTC. At time intervals of 1, 3, 6, and 12 hours, I removed a tray from each of the four cabinets. Thus, after one hour I removed one tray of fry from each of the 0, 100, 250, 500, 1000 and 2000 ppm solutions and drained it of OTC. After draining, I placed these trays in a cabinet supplied with flowing hatchery water. I repeated this process after 3, 6, and 12 hours. During the exposure I monitored dissolved oxygen, temperature and pH in the different reservoirs.

Initially I did two exposures approximately one month apart. The first consisted of five concentrations: 0, 100, 250, 500 and 1000 ppm and the second exposure consisted of 0, 100, 250, 500 and 2000 ppm. Reported here



are results of the 100, 250, 500 and 2000 ppm groups from the second exposure and the 1000 ppm group from the first exposure. I combined groups from these two different exposure times to have a linear progression in the concentrations from the control (zero ppm OTC) to 2000 ppm. It should be noted that the 1000 ppm group did come from a different time exposure in which the physical factors (ie. temperature, pH and dissolved oxygen) were different from those of the second exposure groups.

After completion of the exposures I thoroughly flushed all trays and returned them to their respective cabinets. At this time I inspected each of the trays for mortality. I then restored the flow of hatchery water and drained all of the OTC from the reservoirs. I allowed the exposed fry groups to develop further for 45 days, to ensure that all of the OTC taken up by the fry during exposure could be assimilated into the endoskeletons or excreted. I removed any silt that accumulated in the Heath trays by flushing the trays with stream water.

The unexposed control group of fry may have become contaminated with OTC accidentally during the fresh water flushing of the exposed groups. I inadvertently allowed the exposed fry trays to be stacked on top of the unexposed group during the final fresh water flushing. The residual OTC left in the exposed fry trays could have been enough to

contaminate the unexposed fry. I collected fry from the following brood year which were unexposed to OTC and used them as the unexposed fry group. The fry I used for the unexposed group came from the same NOPAD S40LZ incubator as the fry of the exposed group, but undoubtedly had a different genetic makeup. The fry used for the control group were the same weight and length as those of the exposed groups.

After the 45 day period, I estimated the number of subsequent mortalities from each of twenty groups and then sacrificed the fry. I took a random sample of ten fry from each of the groups and weighed and measured them. I placed the remainder of the 250 fry from each group in plastic bags, froze them at  $-12^{\circ}\text{C}$  and labeled these plastic bags according to the concentration and duration of exposure.

#### SPECTROFLUOROMETRIC ANALYSIS

I removed ten specimens from each of the twenty-four groups and dissected the sagitta otoliths from each with fine forceps under threefold magnification. I stored the otoliths in 1.5 ml test tubes at  $-12^{\circ}\text{C}$ . These vials do not add to the fluorescence of a sample.

I used 5% trichloroacetic acid (pH 0.90) to dissolve the otolith pairs and to release the OTC calcium complex into solution. TCA is known to have a low background

fluorescence when used to dissolve tetracycline and subsequently buffered to pH 9.5 (Kohn 1961). Because TCA is not stable for long periods of time at low concentrations, (Windholz et al. 1983) I prepared a 30% stock solution of TCA with deionized water and Sigma anhydrous TCA crystals (Mol. Wt. 163.4). From this 30% stock solution I prepared 5% solutions to dissolve the pairs of otoliths. I pipetted 0.15 ml of 5% TCA into the vials containing the pairs of otoliths. I then sealed the vials and shook them gently to completely submerge the otoliths in the TCA. I put the vials in a dark refrigerator to dissolve overnight. The otoliths completely dissolved within twelve hours.

I used a 0.01 M glycine buffer to maintain the otolith solutions at a desired pH of 9.5. The glycine buffer is made in two stages. Stock solution A is made by dissolving 11.26 grams of (0.10 mole) glycine, 5.85 grams (0.1 mole) NaCl, and 1.47 grams (0.013 mole) CaCl<sub>2</sub> in water to a final volume of 1.0 liter. Stock solution B is 1.0 M NaOH. The working glycine buffer consists of 800 ml of stock solution A, 56.6 ml of stock solution B, and added water to 1.0 liter final volume. The test pH of the solution should be 9.5 (Stekoll et al. 1986). This glycine buffer adds no background fluorescence to the overall sample.

To each of the TCA otolith solutions I added 0.8 ml of glycine buffer and shook thoroughly. Five percent TCA is a strong acid. The glycine buffer was not strong enough to bring the pH of the solution to an optimal pH for OTC fluorescence of 9.5. In order to bring the pH of the total solution up to pH of 9.5, I added 0.05 ml of 1N NaOH to each of the samples. This amount of base consistently brought the overall pH of the final otolith solution to 9.5. The 1N NaOH adds no background fluorescence to the sample. Once I added the 1N NaOH to the samples I shook them thoroughly and allowed them to settle for 20 minutes at room temperature in the dark. At high pH there is a high tendency for light to accelerate decomposition of the OTC. Care must be taken to avoid over-illumination during analysis (Ibsen et al. 1963).

I used a Perkin-Elmer model 650-10S spectrophotofluorometer to analyze the otolith samples. The spectrophotofluorometer is designed for the measurement of fluorescence at limited emission wavelengths and limited excitation wavelengths. I set the excitation wavelength at 370 nm and the emission wavelength to 515 nm (Stekoll et al. 1986). I set both the excitation and emission slit widths to 5 nm and measured fluorescence with a warm machine. Because the machine is sensitive it must be allowed to warm up for an hour so that the xenon bulb will

be at a constant temperature while the samples are being analyzed.

I poured each sample into a quartz cuvette and analyzed it in the spectrofluorometer. Each sample requires approximately three minutes for analysis. After I read one sample I emptied it into a waste beaker and thoroughly rinsed the quartz cuvette with distilled water. I converted the digital reading of the spectrofluorometer to fluorescence by dividing the digital reading by the sensitivity setting of the machine (i.e. a reading of 45 at a sensitivity setting of 30 would translate to fluorescence units of 1.5).

I analyzed a given concentration group on the same day. I did this to ensure the accuracy of the readings. The chemicals being used (i.e. 5% TCA and 0.10 M glycine buffer) are unstable at room temperature. To ensure a consistency throughout a given concentration group I analyzed that group in one day.

I used a standard curve to quantify the amount of OTC present in each otolith sample. The reagent blank consisted of 0.15 ml (5% w/v TCA), 0.80 ml (glycine buffer), and 0.05 ml (1N NaOH) (total volume 1.0 ml). The standards ranged in concentrations from 0.01  $\mu\text{g}$  OTC/ml to 1.0  $\mu\text{g}$  OTC/ml. I analyzed a new standard for each of the concentrations and used the mean reading of these six standards



for the standard curve used to estimate the amount of OTC present in the otolith sample.

#### STATISTICAL ANALYSIS

I used the Bell Labs S data analysis system to analyze my resulting data (Becker and Chambers 1984). The analyses I performed included scatterplots of the concentrations and durations of exposure, notched box plots of concentrations, and durations of exposure, and regression analysis. I re-expressed the fluorescence units of each sample to natural logarithms. The notches in a notched box plot define a confidence interval around the median that has been adjusted to make it appropriate for comparisons of two boxes. If the intervals of two boxes do not overlap, then we can be confident at about the 99% level that the two population medians are different (Velleman and Hoaglin 1983). I used straight line regressions (Kleinbaum and Kupper 1978) to analyze the relationship between fluorescence and duration of exposure and concentration.

## RESULTS

## RESPONSE TO TREATMENT

During the OTC exposure I monitored dissolved oxygen, water temperature, and pH in the different reservoirs (Table 1). The dissolved oxygen in all of the concentration reservoirs gradually decreased, but was within the limits of tolerance for pink salmon. The pH in each of the concentration reservoirs remained constant, only changing by a few tenths in each reservoir. The temperature in all the reservoirs increased gradually during the 12 hour duration of exposure. However, the temperatures were within the tolerance levels for pink salmon.

The mean weight of the fry was 0.21 grams and the mean length was 24 millimeters. Stronger concentrations of OTC caused the fry to be more lethargic. Before addition of the OTC to the reservoirs fry could be seen swimming at the surface of the Heath trays. Once I added the OTC to the 2000 ppm group the water became too cloudy to see what effects the OTC was having on the fry. After fifteen minutes I could no longer see fry in the 2000 ppm treatment swimming at the surface. In 1000 ppm fry laid on their sides, ventilating rapidly and did not respond to stimulus of a flashlight. The fry in 500 ppm could be observed clearly and appeared stressed. These fry did not respond



Table 1. Water chemistry of reservoirs. Recorded here are the dissolved oxygen, pH and temperature of the five concentration reservoirs. Readings were taken at one six, and twelve hours. Missing values are represented by (\*).

CONCENTRATION	HOURS EXPOSED	DISSOLVED O <sub>2</sub> (Mg/L)			pH			TEMPERATURE °C		
		1	6	12	1	6	12	1	6	12
100 PPM		11.9	11.2	10.7	7.4	7.8	7.4	3.6	4.6	5.1
250 PPM		11.7	11.1	10.6	7.3	7.8	7.4	3.7	4.7	5.3
500 PPM		11.7	11.0	10.5	7.5	7.9	7.8	3.7	4.6	5.4
1000 PPM		*	13.9	13.4	7.6	*	7.4	2.1	2.9	3.6
2000 PPM		11.6	10.8	10.4	7.6	7.9	7.9	3.8	4.8	5.5

to light stimulus, ventilated rapidly and were unable to maintain their equilibrium, resulting in them drifting against the upper screen of the trays with the effluent water. In 250 ppm the water was clear and the fry appeared alert, swam upright and ventilated normally. However, the response of fry in 250 ppm to light stimulus was slower than that of the control fry. I found no behavioral differences between the unexposed fry and the fry in 100 ppm.

As the experiment progressed for two and three hours, the fry exposed to higher concentrations began to recover. Fry exposed to 500 and 1000 ppm started to return to an upright swimming position and ventilated less rapidly. Fry exposed to 2000 ppm appeared stressed all through the exposure.

At the end of the exposure I found all of the fry exposed to 2000 ppm for twelve hours dead. I found 70% mortality in fry exposed to 2000 ppm for six hours. Surviving fry of this exposure appeared weakened, swam irregularly and displayed poor equilibrium. Fry in all other concentrations and durations survived and exhibited normal behavior after being restored to clear water. They ventilated normally, responded to light stimulus and swam upright.

The standard curve used to estimate the actual amount of OTC present in each otolith was linear from the blank

(0.0  $\mu\text{g}$  OTC/ml) to 0.50  $\mu\text{g}$  OTC/ml (Figure 1). Although the standard ranged in concentration from 0.01  $\mu\text{g}$  OTC/ml to 1.0  $\mu\text{g}$  OTC/ml; the upper range of 1.0  $\mu\text{g}$  OTC/ml is not plotted in Figure 1 because none of the otoliths fluorescence reached near that range of concentration. The lowest amounts of OTC present in the fry otoliths was shared by both 100 and 250 ppm exposed for one hour. Both of these groups had 0.0018  $\mu\text{g}$  OTC/otolith pair. The highest amount of OTC present in the fry otoliths was in the group exposed to 500 ppm for twelve hours. This group had 0.0765  $\mu\text{g}$  OTC/otolith pair.

In two instances during the 45 day assimilation period, due to freshets the top trays in each cabinet became filled with silt. This silt almost completely restricted the water flow. On both of these occasions I drained the trays of silt and restored complete water flow to the cabinets. None of the fry died from these two silting instances.

#### OTC UPTAKE vs. DURATION OF EXPOSURE

Scatterplots of fluorescence versus the 100 ppm showed an increase of OTC uptake with duration of exposure from zero to twelve hours (Figure 2). In 250 ppm the fluorescence increased up to exposures of three hours. However, after three hours of exposure there was a decrease

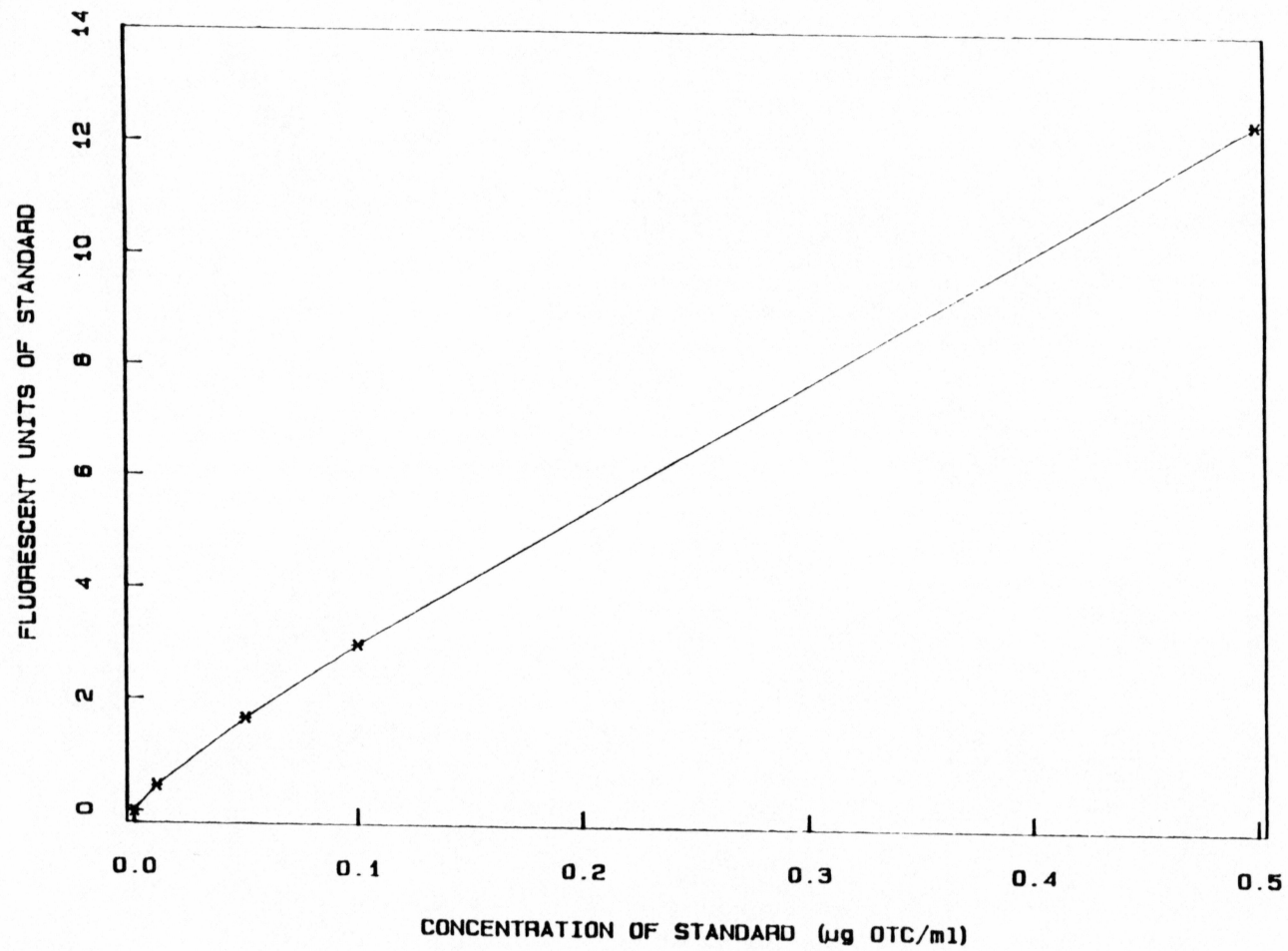


Figure 1. Standard curve of oxytetracycline dissolved in 5% TCA (w/v). The total volume of the sample equals 1.0 ml.

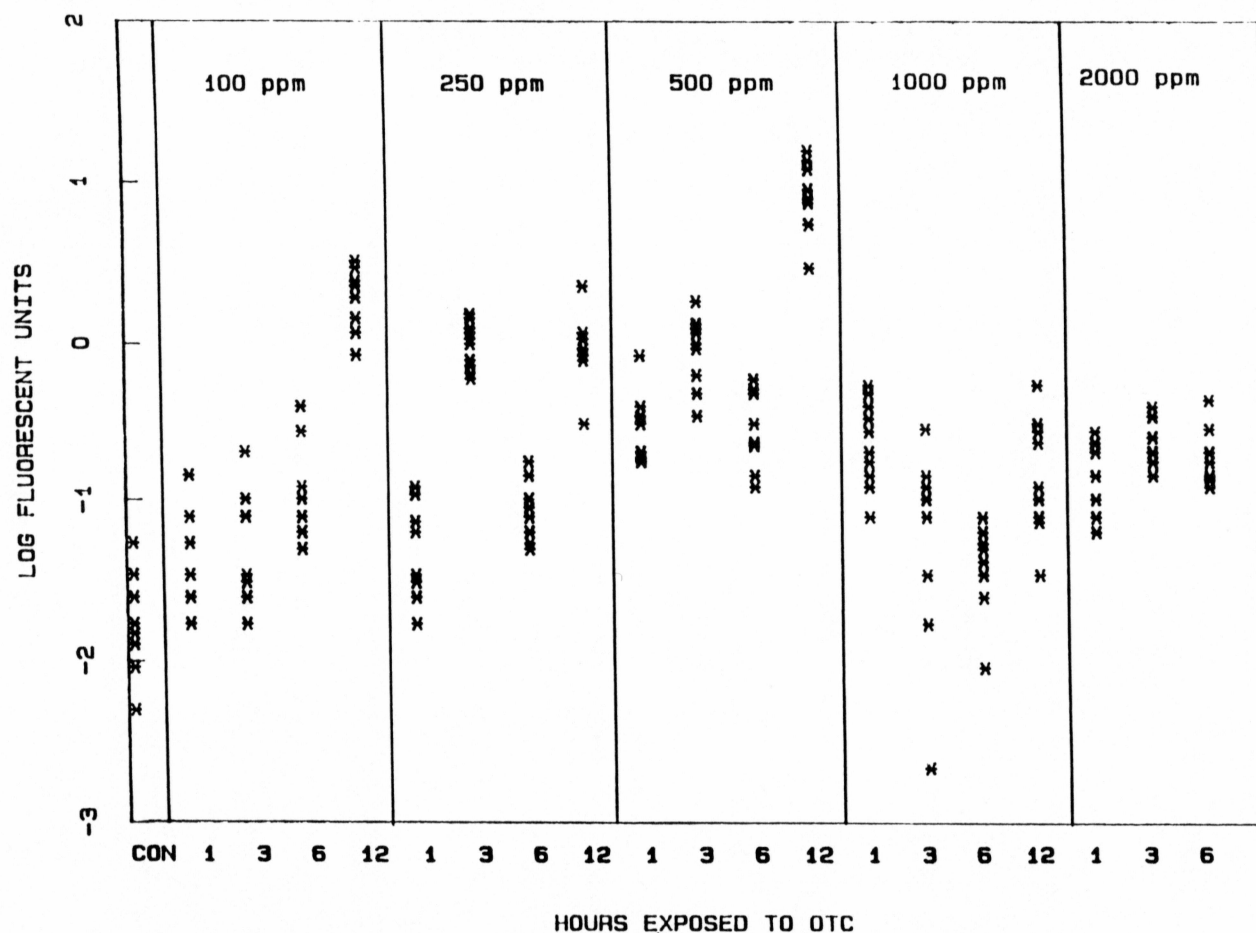


Figure 2. Scatterplots of paired otolith fluorescence vs. duration of exposure within the same concentration. N=10 per duration of exposure, and the durations of exposure to OTC range from the control to twelve hours. The concentrations range from 100 ppm to 2000 ppm with the control at the far left of the graph. Note the nonlinearity of the x-axis.

in fluorescence, and after twelve hours of exposure, fluorescence returned to the three hour level. The scatterplot of the 500 ppm group has a pattern similar to the 250 ppm groups. Fluorescence increases up to three hours of exposure, declines after six hours of exposure, and after twelve hours of exposure increases to the highest value in the 500 ppm concentration. I found no distinguishable difference in fluorescence between durations of exposure in 1000 ppm, however, all groups demonstrated greater fluorescence than did the unexposed control fry. The 2000 ppm group has a sharp increase in fluorescence from unexposed to the one hour duration, however, after three or six hours duration there is no increase in OTC uptake. The mean amounts of OTC present in each otolith pair for the different exposures with the same concentration are plotted in Figure 3. The amounts of OTC present per otolith pair were estimated with the standard curve.

The trends mentioned above are easier to see as notched box plots of the data (Figure 4). At 100 ppm all but the one hour exposure had more OTC per otolith pair than did the unexposed control group. The otoliths of the three and six hour exposures had significantly greater OTC than did the unexposed but were not significantly different from each other. Twelve hours of exposure to 100 ppm produced significantly higher OTC concentration than did



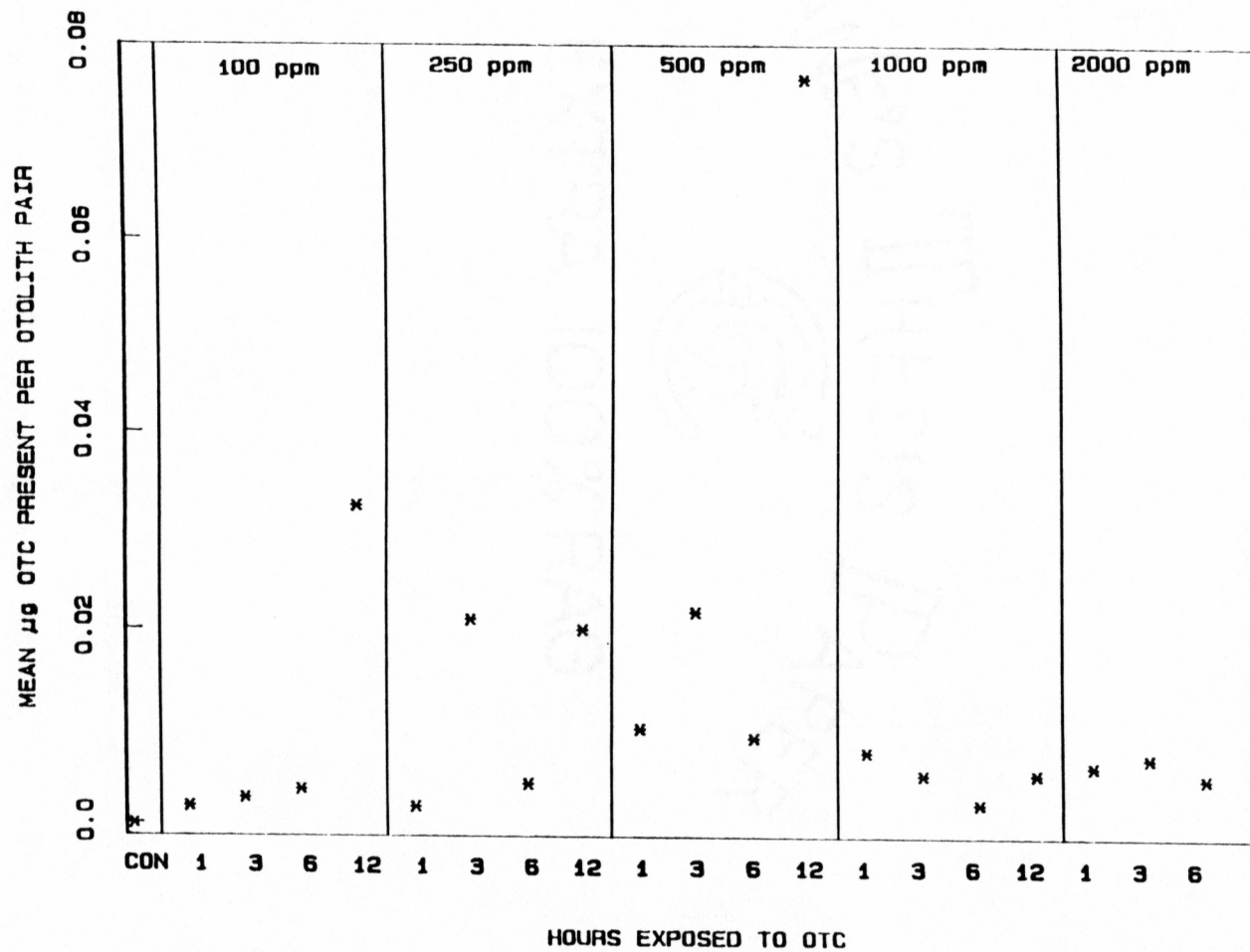


Figure 3. The mean amounts of OTC present in each otolith pair for the different exposures within the same concentration. Values are estimated using the standard curve. Note the nonlinearity of the x-axis.



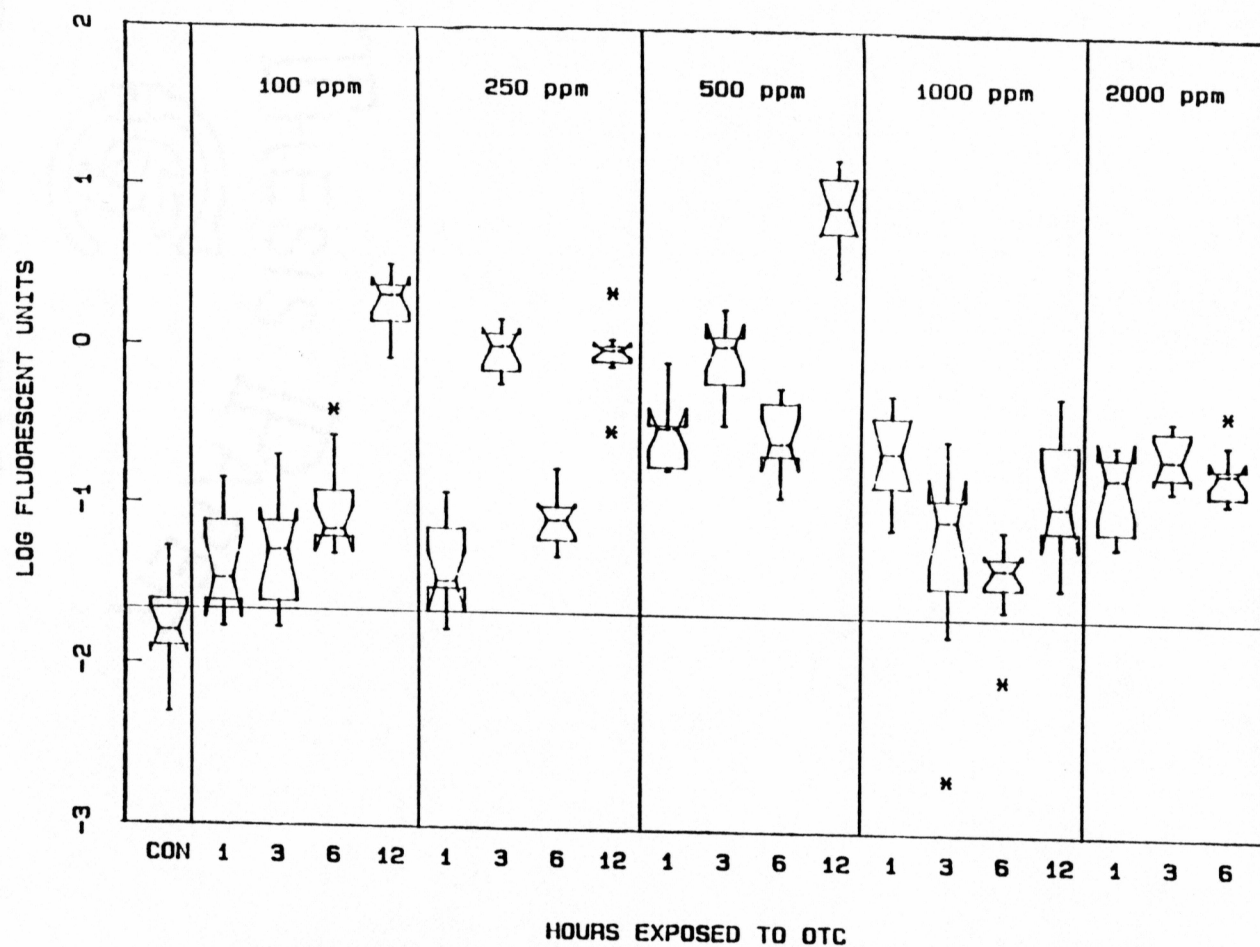


Figure 4. Notched box plot of paired otolith fluorescence vs. duration of exposure in the same concentration. N=10 per duration group, and the durations range from one to twelve hours, with the control at the left of the figure. The median confidence interval equals 99%. Note the horizontal line identifying the significant difference of other box plots from the control. Note the nonlinear x-axis.

all the other times of exposure to 100 ppm.

One hour exposure to 250 ppm produced significantly higher incorporated OTC than did no exposure. Three hours of exposure produced significantly more OTC in otoliths when compared to one hour and shares the highest overall incorporation with twelve hours of exposure. Six hours of exposure, on the other hand, produced significantly less OTC uptake than three or twelve hours, but was still greater than that of the control or the one hour duration of exposure.

All of the time exposures in the 500 ppm produced significantly greater OTC concentration in the otoliths than occurred in the control group. The twelve hour exposure produced the greatest OTC uptake of all the exposure groups. However, six hour exposure resulted in significantly less OTC incorporation than three hour exposure, making it equal to the OTC uptake after one hour of exposure.

All four time exposures to 1000 ppm showed significantly more OTC uptake than the unexposed control group, but there is no significant difference among them. Exposure to 2000 ppm produces a pattern similar to that of 1000 ppm. All three durations of exposure to 2000 ppm (one, three, and six hours) showed significantly more OTC incorporation than the unexposed fry, however, none of

these durations differ significantly from each other.

Straight line regressions of otolith exposure times in the same concentration showed the six hour time exposure fell below the regression line in many of the concentration groups. This OTC uptake deficiency at the six hour exposure increases with increases in concentrations from 100 to 2000 ppm. The 500 ppm group had the highest slope ( $1.68 \times 10^{-1}$ ) and the 1000 ppm group had the lowest slope of the four concentration groups ( $3.15 \times 10^{-2}$ ). Table 2 shows the regression summaries of the concentration groups.

#### OTC UPTAKE AS A FUNCTION OF IMMERSION CONCENTRATION

Scatterplots of OTC concentrations in otoliths exposed for one hour showed increased OTC uptake to 500 ppm (Figure 5). OTC uptake in otolith pairs then declined at concentrations of 1000 and 2000 ppm exposed for one hour. For the three hour exposure OTC uptake increases to the 500 ppm exposure and then declines as the concentrations increase to 1000 and 2000 ppm.

During the six hour exposures there was an increase in OTC uptake to 500 ppm exposure and a lesser uptake at greater concentrations. A slight increase in otolith OTC occurs at 2000 ppm, but not to that of the 500 ppm exposure. Fry exposed to OTC for twelve hours show the same pattern of increasing uptake up to 500 ppm and less

Table 2. Regression summaries of the concentration groups. Included in the table is significance of the regression slopes from zero at the 0.001 level.

	SLOPE	INTERCEPT	$r^2$	N	F-VALUE	Df		SIG. DIFF. FROM ZERO @ 0.001 LEVEL
						N	D	
100 Mg/L	1.587 e -1	-1.736	0.817	50	215.48	1	48	YES
250 Mg/L	1.113 e -1	-1.347	0.426	50	35.63	1	48	YES
500 Mg/L	1.676 e -1	-1.132	0.656	50	91.70	1	48	YES
1000 Mg/L	3.147 e -2	-1.330	0.063	50	3.22	1	48	NO
2000 Mg/L	1.357 e -1	-1.330	0.377	40	22.97	1	38	YES

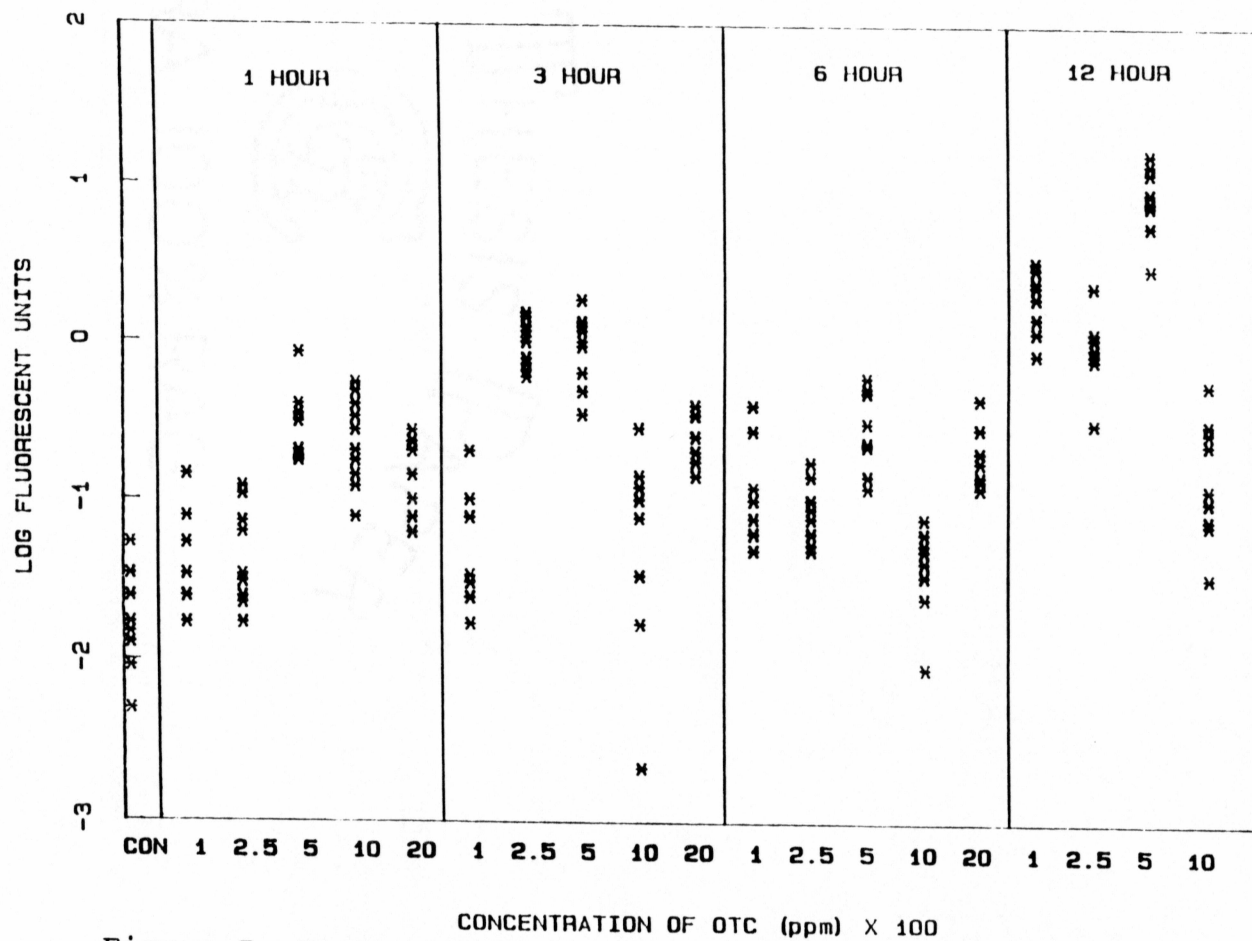


Figure 5. Scatterplots of paired otolith fluorescence vs. concentration within the same duration of exposure. N=10 per concentration group and the concentrations range from the control to 2000 ppm. The durations of exposure range from one hour to twelve hours, with the control at the far left of the graph. Note the nonlinearity of the x-axis.



uptake for greater concentrations. There are some anomalies in the twelve hour exposures; the 100 ppm group had a higher level of OTC than did the 250 ppm group. The fry in the 2000 ppm exposure group all died from the long exposure so there is no representation for them in this scatterplot. The mean amounts of OTC taken up into each otolith pair for the different concentrations within the same exposure is plotted in Figure 6. The amounts of OTC present in each otolith pair was estimated using the standard curve.

I also used notched box plots to measure highly significant (99% level) differences among concentrations within the same duration of exposure (Figure 7). One hour exposure to 100 and 250 ppm did not produce significantly greater OTC uptake when compared to the control. Exposure to 500 ppm produced significantly more OTC incorporation than no exposure, or exposure to 100 and 250 ppm. Exposure to 1000 ppm produced no significant increase in otolith OTC over exposure to 500 ppm. The OTC uptake in response to 2000 ppm is not significantly different from the response to 500 or 1000 ppm.

Three hour duration of exposure to all the concentrations of OTC produced significantly more uptake than did no exposure. There was a large increase in the amount of otolith OTC from the control to 100 and 250 ppm. Exposure



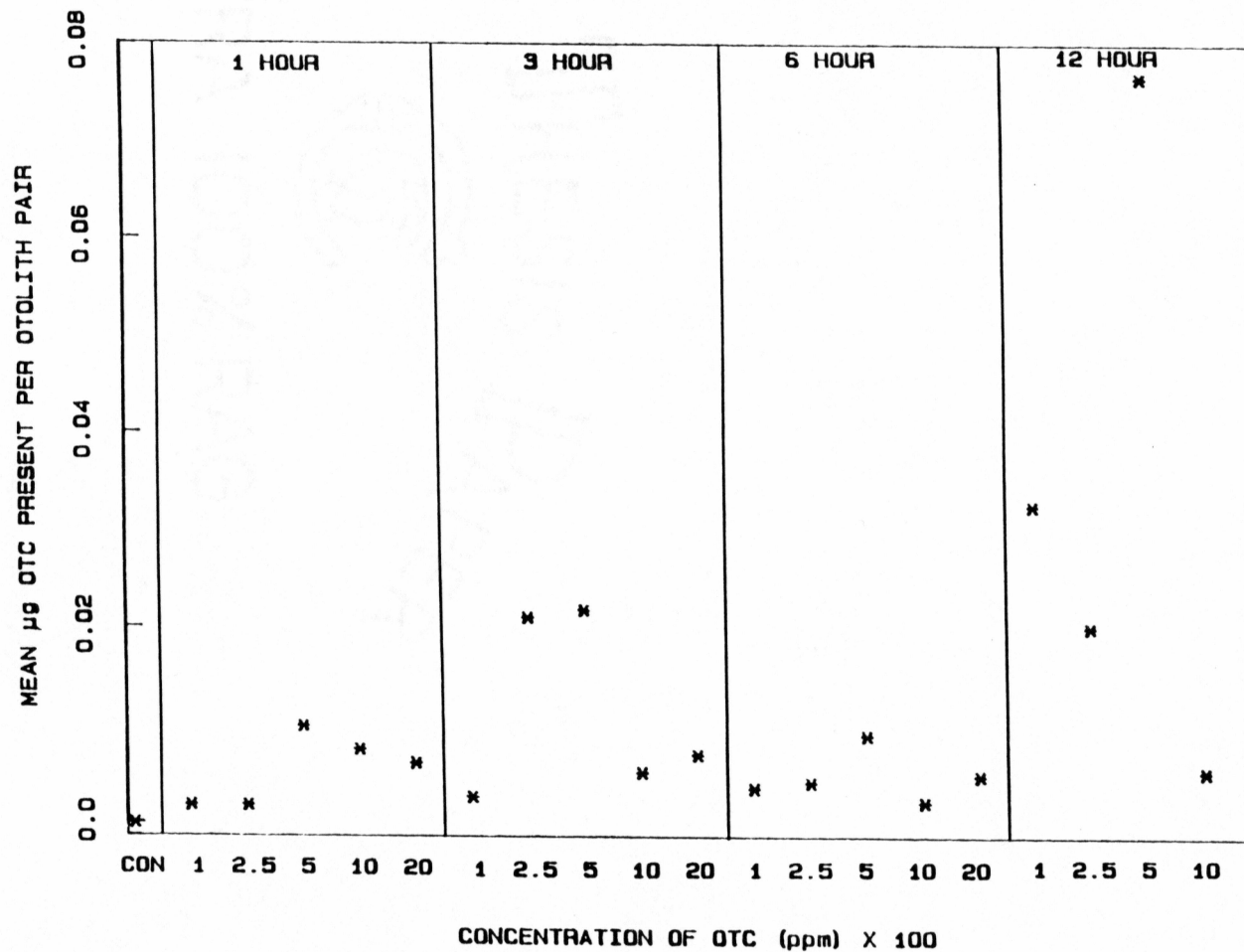


Figure 6. The mean amounts of OTC present in each otolith pair for the different concentrations within the same duration of exposure. Values are estimated using the standard curve. Note the nonlinearity of the x-axis.

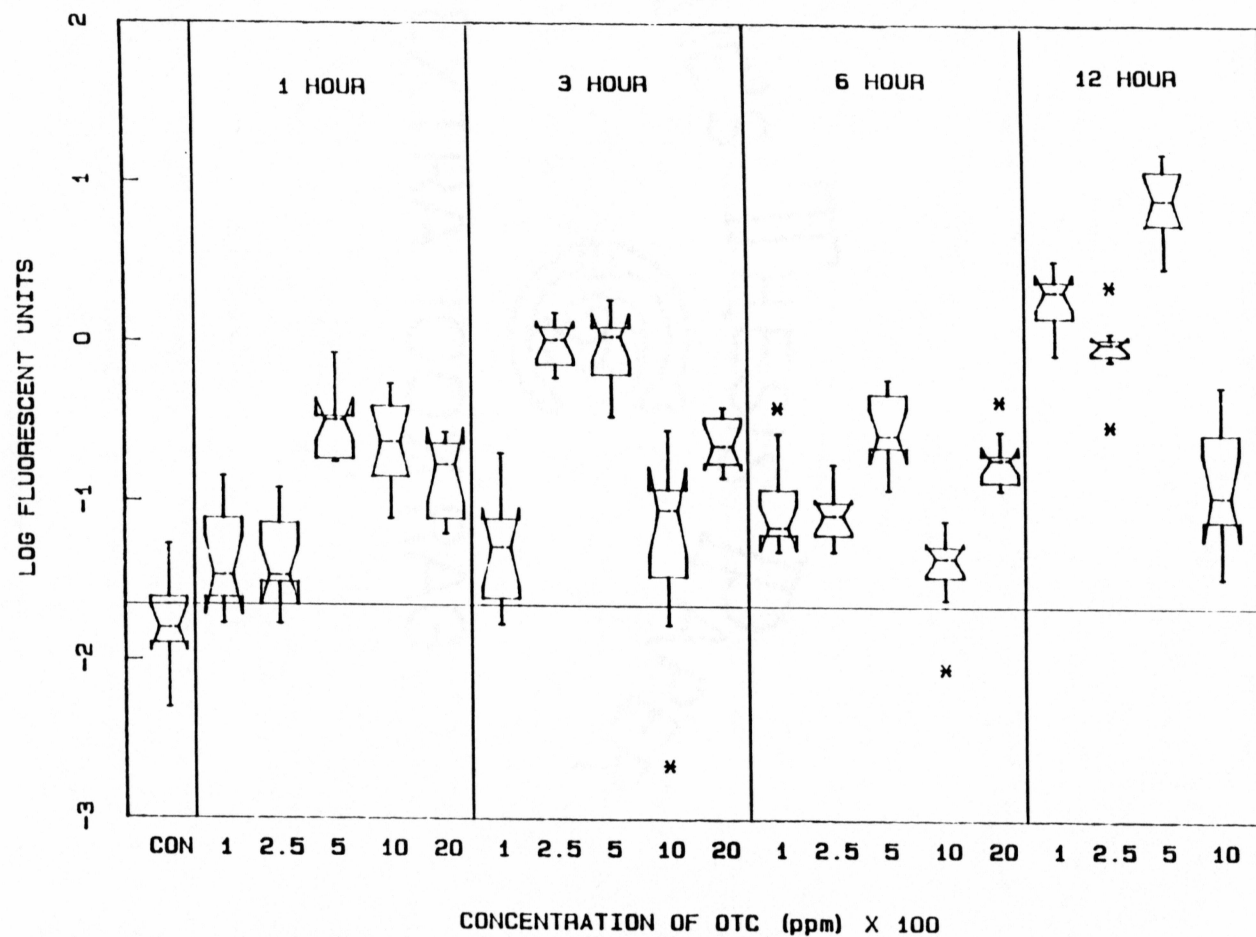


Figure 7. Notched box plot of paired otolith fluorescence vs. OTC concentration within the same duration of exposure. The median confidence interval equals 99%. Box plots are grouped into duration of exposure with the control at the far left of the figure. Note the horizontal line identifying the significant difference of the other box plots from the control. Note the nonlinear x-axis.

to 250 and 500 ppm produced no significant difference in OTC uptake. Exposure to 1000 ppm produced significantly less OTC in the otoliths than exposure to 250 and 500 ppm, but not significantly different from exposure to 100 ppm. Exposure to 2000 ppm produced less otolith OTC than exposure to 250 or 500 ppm, but greater than to 100 or 1000 ppm.

The pattern of response in the six hour duration of exposure was similar to that produced in the three hour exposure. Each concentration of OTC produced significantly more OTC uptake than did no exposure. There was no significant difference between OTC incorporation produced by 100 and 250 ppm. Exposure to 500 ppm for six hours showed significantly more uptake than to 100 or 250 ppm. Once again there was significantly less OTC uptake from exposure to 1000 ppm for six hours, less than to 100 and 250 ppm exposures but more than the unexposed group. Exposure to 2000 ppm produced increased OTC uptake above exposures to 100 and 250 ppm but not significantly more than to 500 ppm.

Twelve hour duration at any concentration produced significantly greater fluorescence than did the unexposed control. Exposure to 250 ppm for twelve hours produced less fluorescence than exposure to 100 ppm for twelve hours. Exposure of 500 ppm samples fluoresced more than those exposed to 100 ppm, more than to any other

concentration for twelve hours. Once again exposure to 1000 ppm produced significantly less fluorescence than to 100, 250, or 500 ppm but still significantly more than the control.

The straight line regressions of the concentration groups exposed for the same duration of time shows that the 1000 and 2000 ppm groups consistently fell below the regression line. The amount these two concentrations lag below the regression line increases with increases in duration of exposure. I ran three sets of regressions on the different exposure groups to see what effect the 1000 and 2000 ppm groups have on the data (Table 3). In the first set of regressions, which included all of the concentration groups, the slope of the one hour exposure was the greatest ( $3.875 \times 10^{-4}$ ). The regression slope of the three hour exposure was the lowest ( $1.963 \times 10^{-4}$ ). The regressions excluding the 1000 and 2000 ppm groups show that the twelve hour exposure had the steepest slope ( $4.200 \times 10^{-3}$ ) and the six hour exposure had the lowest slope ( $2.096 \times 10^{-3}$ ). The final set of regressions included only the 100 to 500 ppm groups from each exposure time. In these regressions the three hour time exposure had the greatest slope ( $2.800 \times 10^{-2}$ ) and the six hour exposure had the lowest slope ( $1.320 \times 10^{-3}$ ).

Table 3. Regression summaries of durations of exposure. Represented here are three different regression runs for each duration of exposure. The first group (1.) includes the concentrations from the control to 2000 ppm, the second group (2.) contains the concentrations from the control to 500 ppm, and the third group (3.) includes the concentrations from 100 to 500 ppm.

		SLOPE	INTERCEPT	$r^2$	N	F-VALUE	Df		SIG. DIFF. FROM
							N	D	ZERO @ 0.001 LEVEL
1 HOUR	1.	3.875 e -4	-1.335	0.264	60	20.79	1	58	YES
	2.	2.309 e -3	-1.750	0.678	40	79.94	1	38	YES
	3.	2.230 e -3	-1.723	0.593	30	40.82	1	28	YES
3 HOUR	1.	1.963 e -4	-0.960	0.034	60	02.03	1	58	NO
	2.	3.600 e -3	-1.545	0.698	40	87.96	1	38	YES
	3.	2.800 e -2	-1.249	0.520	30	30.34	1	28	YES
6 HOUR	1.	2.400 e -4	-1.246	0.123	60	08.10	1	58	NO
	2.	2.096 e -3	-1.545	0.608	40	58.82	1	38	YES
	3.	1.320 e -3	-1.249	0.405	30	19.09	1	28	YES
12 HOUR	1.	2.480 e -4	-0.387	0.008	50	00.41	1	48	NO
	2.	4.200 e -3	-1.056	0.621	40	62.18	1	38	YES
	3.	1.700 e -3	-0.117	0.457	30	23.59	1	28	YES

## DISCUSSION

## SUPPORT OF PREVIOUS WORKS

The results of the data show that pink salmon fry otoliths can be tagged with OTC immersion and that OTC can be detected with fluorescence spectrophotometry. All but two of the box plot groups (100 ppm and 250 ppm exposed for one hour) had significantly higher fluorescence than did the control group (Figures 4 and 7).

These findings are consistent with past OTC marking experiments. Weber and Ridgway (1967) reported good visual OTC marks in bones of Oncorhynchus species fed 250 mg/kg body weight OTC for four days. Odense and Logan (1974) acquired similar results in Atlantic salmon by feeding 250 mg/kg body weight per day for five days. These doses provided a stable OTC mark which was readily visible in the centrum when the bone was cleaned and examined under long-wave ultraviolet light. Odense found that the fluorescent marks persisted for 23 months. Hettler (1984) successfully marked larval spot Leiostomus xanthurus and pinfish Logodon rhomboides otoliths in immersions of tetracycline. Hettler was able to achieve 50 to 100% marking at concentrations of 250 and 500 mg/l tetracycline at exposures of one to two hours.

Although I exposed several groups of fry to high



concentrations of OTC (500, 1000 and 2000 ppm) these concentrations would not be recommended in most hatcheries. High concentrations of 500, 1000 and 2000 ppm OTC produce signs of stress in the fry exposed to them. The majority of these higher concentrations did not produce higher amounts of OTC in the otolith when compared with the lower concentrations (Table 4). The higher stocking densities of a hatchery would probably magnify the amount of stress put on the fry due to competition for oxygen and the removal of metabolic wastes. Even though only the fry exposed to 2000 ppm for six and twelve hours suffered mortalities in my experiment, the combination of higher stocking densities and stress caused by higher levels of OTC could result in high fry mortality at concentrations of 500 ppm and higher.

Fry in 100 and 250 ppm OTC, on the other hand, exhibited little signs of being stressed during OTC exposure. Fry in these concentrations ventilated steadily, exhibited consistent equilibrium and responded to light stimulus. This indicates that fry should be exposed to this range of concentrations. Lower marking concentration will ensure a higher survival rate during tagging.

It is possible that the quantifying of observed behavioral stress could be used to determine at what concentration the fry could be safely tagged with OTC. Sydor et al. (1982) used ventilatory behavior in blue gill Lepomis

Table 4. Estimated amounts of OTC present in fry otoliths. OTC amounts were estimated from standard curve. Background fluorescence was subtracted from gross fluorescence to obtain net fluorescence.

CONCENTRATION GROUP	MEAN GROSS FLUORESCENCE N=10	STANDARD DEVIATION	BACKGROUND FLUORESCENCE REDUCTION	NET FLUORESCENCE	MEAN µg OTC PRESENT
CONTROL	0.177	0.052	0.177	0.000	0.0000
100 PPM 1 HOUR	0.242	0.098	0.177	0.065	0.0018
100 PPM 3 HOUR	0.292	0.102	0.177	0.115	0.0027
100 PPM 6 HOUR	0.327	0.136	0.177	0.150	0.0036
100 PPM 12 HOUR	1.392	0.231	0.177	1.215	0.0325
250 PPM 1 HOUR	0.242	0.079	0.177	0.065	0.0018
250 PPM 3 HOUR	1.027	0.142	0.177	0.850	0.0210
250 PPM 6 HOUR	0.352	0.065	0.177	0.175	0.0042
250 PPM 12 HOUR	1.012	0.204	0.177	0.835	0.0200
500 PPM 1 HOUR	0.627	0.139	0.177	0.450	0.0099
500 PPM 3 HOUR	1.047	0.204	0.177	0.870	0.0218
500 PPM 6 HOUR	0.577	0.142	0.177	0.400	0.0090
500 PPM 12 HOUR	2.472	0.512	0.177	2.295	0.0765
1000 PPM 1 HOUR	0.547	0.147	0.177	0.370	0.0075
1000 PPM 3 HOUR	0.382	0.146	0.177	0.205	0.0052
1000 PPM 6 HOUR	0.272	0.056	0.177	0.095	0.0022
1000 PPM 12 HOUR	0.397	0.164	0.177	0.220	0.0053
2000 PPM 1 HOUR	0.477	0.106	0.177	0.300	0.0061
2000 PPM 3 HOUR	0.537	0.086	0.177	0.360	0.0070
2000 PPM 6 HOUR	0.497	0.929	0.177	0.320	0.0049

macrochirus as a means of assessing environmental water quality. The behavioral signs of stress which I observed that could be used as quantifiers for safe exposure were the respiratory rate, righting responses and response time to light stimulus. Quantifying the qualitative responses to OTC exposure could be a means of accurately assessing safe concentration exposures.

The fry used for the control came from the following year because the original control fry may have become contaminated with OTC. Although it is true that the control group (zero exposure to OTC) is from a different genetic stock, (year class) it is unlikely that the fluorescence of the exposed otoliths will differ significantly from controls of the following year class. Otolith mineralization of fry incubated in the same water supply from different year classes is probably not significantly different.

Otolith OTC uptake increased relative to the control group in all of the concentrations with increased durations of exposure. However, this increase in OTC uptake was not linear from the control group to the twelve hour duration of exposure. There is an apparent drop in OTC uptake in all the concentrations at the six hour duration of exposure. The OTC uptake then increases slightly at twelve hours of exposure, but this increase is not sufficient to

make the relationship between the durations linear again. The reasons for this drop in otolith fluorescence at the six hour durations are unclear.

Assuming that this drop in fluorescence is real, one possible reason for the drop in otolith fluorescence at six hours exposure might be the natural cycling of the fry's circadian rhythm. Campana and Neilson (1985) and others have suggested that growth of fish otoliths are governed by circadian rhythms (once per 24 hours) which are entrained by photoperiod, but susceptible to modification by other cyclic environmental variables. Campana's findings are consistent with Mugiya et al. (1981) where evidence of a circadian rhythm of calcium uptake in goldfish was presented. Uptake of labeled calcium ceased near dawn each day and was associated with reduced plasma levels of calcium and concurrent reduction in deposition on the goldfish otolith. This cyclical reduction in calcium uptake could be the contributing factor in the reduction of otolith fluorescence at the six hour exposure. The fry could have experienced a natural low cycling of their circadian rhythm which reduced the deposition of calcium/OTC on the otolith.

Another possible explanation for this drop in fluorescence at the six hour duration of exposure could be induced stress. Experiments in which young coho salmon

(Oncorhynchus kisutch) were stressed while immersed in labeled calcium water demonstrated that check formation was associated with reduced calcium deposition on the otolith (Campana 1983). If the reduction in otolith calcium deposition is proportional to stress level, then the calcium protein ratio will be reduced accordingly in the corresponding growth increment (Campana and Neilson 1985). Campana's findings that stressed coho salmon have reduced calcium deposition on the otolith might also explain the drop in otolith fluorescence at the six hour duration in my experiment. Because of stress from the length of time exposed the amount of OTC being absorbed from the ambient water was reduced. This might explain the decrease in fluorescence of some of the concentration groups at the six hour duration of exposure, and the lack of recovery at the twelve hour duration of exposure.

One other explanation for this drop in otolith fluorescence could involve the uptake and deposition of OTC into the otolith once it is in the fry's body (Figure 8). This idea can be explained by using a model with a series of constants which represent the rate at which OTC is moved from the ambient water and through the fish's body to the otolith. The constants are defined as follows; 1)  $K_1$  is the rate of uptake of OTC from the ambient water into the fry's body, 2)  $K_2$  is the rate at which the pooled body OTC



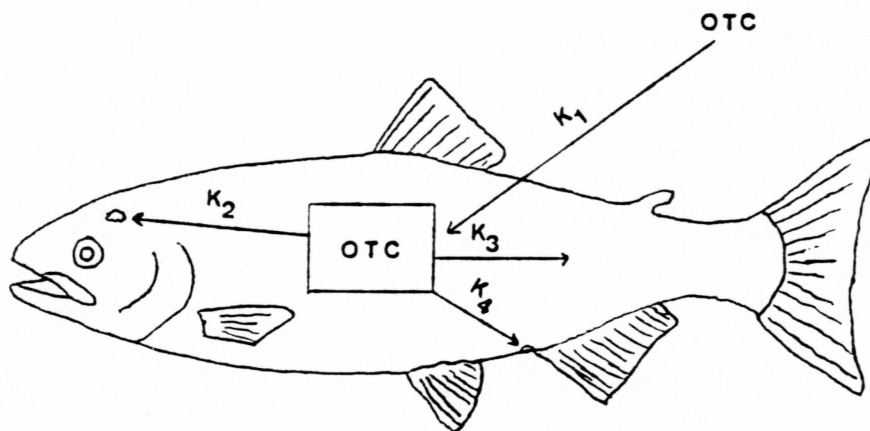


Figure 8. Schematic diagram of uptake and deposition of OTC from the ambient water into the fry's otoliths and other body parts.



is absorbed into the otolith, 3)  $K_3$  is the rate at which the pooled body OTC is absorbed into other tissues, 4)  $K_4$  is the rate at which OTC is eliminated from the body.

If we assume that  $K_2$  (the rate at which the pooled OTC is absorbed into the otolith) and  $K_3$  (the rate at which OTC is absorbed into other body tissues) are the same, and that due to stress or a low cycling of the circadian rhythm as explained previously  $K_1$  (the rate of uptake of OTC from the ambient water) is reduced or stopped, and that  $K_4$  (the rate at which OTC is eliminated from the body) continues or is sped up because of excess stress put on the fry from the OTC, then this combination could result in lower and even less otolith fluorescence at six hours than occurred at the three hour duration of exposure. Due to the combined effects of reduced OTC uptake and continued OTC elimination from the body an overall reduction of otolith OTC would be produced. This model would explain why the otolith fluorescence of the six hour durations of exposure is not equal to the three hour duration as would be expected, and is actually less than the three hour durations of exposure.

In all of the time exposure groups (one, three, six and twelve hours) otolith OTC increased steadily with increases in concentration up to 500 ppm. At this point there is a decrease in otolith OTC. The otolith OTC remains low to 2000 ppm. I believe the reason for this

drop in otolith fluorescence is stress associated with the higher concentrations of OTC. The fry appear to reach a concentration saturation point above which they do not assimilate as much OTC from the ambient water. Lower levels of calcium/OTC plasma levels result in lower otolith OTC. The OTC that does occur in the otoliths at the 1000 and 2000 ppm levels is a result of the sheer concentrations of those groups.

The slopes of the exposure regressions show which is the best exposure time for marking the fry otoliths (Table 3). The regression slopes of the three hour exposures is the greatest for regression group three. This shows that the efficiency of OTC absorbance by the fry might be optimized at three hours of exposure.

There is an equal return of otolith fluorescence for invested OTC concentration from the control to 500 ppm (Table 3). This is evidenced by the  $r^2$  values of the regressions with and without the 1000 and 2000 ppm groups included. The  $r^2$  values of the regressions without the 1000 and 2000 ppm groups are closer to one than those including these two groups. The 1000 and 2000 ppm groups have a negative influence on the slopes of the regressions which results in a situation where  $x$  no longer accurately predicts  $y$ . I believe the reason for this is at 1000 and 2000 ppm the fry reach a stress saturation point where they

do not actively absorb as much OTC from the ambient water and the resulting otolith OTC is reduced. Only the one hour exposure which included the 1000 and 2000 ppm groups had a slope significantly greater than zero at the 0.001 significance level. The regression slopes of the three, six and twelve hour exposures which included the 1000 and 2000 ppm group were not significant from zero at the 0.001 significance level.

The  $r^2$  values of the regressions excluding the control did drop in each exposure when compared to the regressions including the control. However, the degrees of freedom of these groups was also less than the groups including the control, and all of the regression slopes of the exposure times excluding the control were different from zero at 0.001 significant level. It is evident therefore that the control is probably not a high leverage point in the exposure groups.

Given the above information alone, it would appear that 500 ppm OTC would be the best concentration to tag pink salmon fry otoliths. However, the behavioral observations indicate that 500 ppm OTC could be too stressful to safely tag high densities of pink salmon fry. The fry in 250 ppm OTC showed little signs of being stressed while at the same time incorporated significant amounts of OTC in their otoliths at three and twelve hours of exposure. The

optimal tagging concentration when considering the combined factors of biological stress and maximum incorporation of OTC in the otolith is probably between 250 and 300 ppm.

Perhaps exposure for shorter periods of time would maximize the amount of OTC taken up by the fry. Exposures of three hours in 250 and 500 ppm OTC produced significant amounts of otolith OTC. Exposing the fry for longer periods of time might result in diminishing returns of OTC incorporation. This would be brought about from long term exposure stress and the degradation of incorporable OTC in the ambient water.

One possible method of tagging which would take advantage of the previously stated phenomenon would be to expose the fry to moderate concentrations of OTC (250 to 300 ppm) for short periods of time (3 hours). After exposure, the fry would be allowed to recover from the stress of being exposed for a period of time in fresh water. After recovery the fry would again be exposed to OTC for the same period of time and at the same concentration. This exposure and recovery cycle would be continued in series until sufficient OTC has been incorporated into the fry's otolith.

#### MARKING COST

The following is a short scenario of the cost to mark pink salmon fry with OTC. Given that OTC costs \$20.00/100 gm, and 1031, 0.22 gram, fry can be exposed to 300 ppm in a cubic foot of water (stocking density 0.5 lb./cu.ft.); then a total of 8.48 grams of OTC is needed to mark the 1031 fry. The cost of marking would be 0.16 cents per fry. This figure is based on the cost of tagging only and does not include technician time needed to administer OTC, or the cost of recovery and analysis of otoliths.

#### TAGGING ADULTS

The possibility of recovering OTC tags from adult pink salmon is a viable idea. Stekoll et al. (1986) developed a method capable of determining levels of OTC in adult salmon otoliths as low as 0.030 to 0.10  $\mu$ g per otolith pair depending on the background interference. The problem in detecting OTC in adults lies in the increased otolith to OTC ratio. Increased otolith tissue means increased background fluorescence in a sample. This background fluorescence is variable from otolith to otolith which makes it difficult to ascertain the actual amounts of OTC present in the otolith. Stekoll et al. (1986) found that the mean background fluorescence in paired adult pink salmon otoliths extracted in 1 M HCl was 0.223 log fluorescent units.



This value corresponds to the OTC present in the fry otolith exposed to 100 ppm for twelve hours which is the second most fluorescent otolith group in my experiment.

The key to accuracy in detecting OTC in adult otoliths is reducing the background fluorescence or increasing the amounts of OTC taken up by the fish as fry. Reduction of the background fluorescence may require more sophisticated OTC extraction procedures or perhaps more sensitive spectrofluorometric equipment. Increasing the amount of exposure appears to be the more likely method of enhancing the possibility of consistently detecting an OTC mark in adult otoliths. One possible method of increasing the amount of OTC absorbed into the fry otolith is the one proposed here which requires a series of exposures to moderate amounts of OTC.



## CONCLUSIONS

Detectable amounts of OTC is bound in pink salmon fry otoliths after immersion. The spectrofluorometer is capable of detecting these quantities of OTC over and above the background otolith fluorescence. Trichloroacetic acid performs well as a decalcifier releasing the OTC into solution, and does not add to the fluorescence of the sample.

The fry are physically stressed when exposed to OTC concentrations of 500 ppm and above. Lower concentrations of OTC should be used to prevent possible high mortality in the higher stocking densities of hatcheries. Two hundred and fifty ppm OTC was the highest concentration to which the fry were exposed without showing marked signs of stress.

The relationship of paired otolith OTC vs. both duration and concentration of exposure was not linear. Concentrations above 500 ppm produced less otolith OTC at most durations of exposure. The nonlinearity of otolith OTC concentration as a function of duration of exposure could be caused by the cyclical uptake of ambient OTC governed by the fry's circadian rhythm.

## LITERATURE CITED

- Becker, R. A. and J. M. Chambers. 1984. S: interactive environment for data analysis and graphics. Bell Telephone Laboratories, Inc., Murray Hill, New Jersey. 550 p.
- Brothers, E. 1985. Otolith marking techniques for the early life history stages of lake trout. Great Lakes Fishery Commission Research Completion Report. 42 p.
- Campana, S. E., and J. D. Neilson. 1985. Microstructure of fish otoliths. Can. J. Fish. Aquat. Sci. 42:1014-1032.
- Campana, S. E. 1983. Calcium deposition and otolith check formation during periods of stress in coho salmon, Oncorhynchus kisutch. Comp. Biochem. Physiol. 75A: 215-220.
- Crandall, K. 1988. Pacific Marine Fisheries Commission. Draft minutes of the 1988 marking meeting. 25 p.
- Gharrett, A. J., S. Lane, A. J. McGregor and S. C. Taylor. 1983. The genetic mark: Its implementation and evaluation. Proc. 2nd N. Pac. Aquaculture Symp. pp. 387-402.
- Hettler, W. F. 1984. Marking otoliths by immersion of marine fish larvae in tetracycline. Trans. Am. Fish. Soc. 113:370-373.
- Ibsen, K. H. and M. R. Urist. 1969. Competition between oxytetracycline and citrate for binding sites on hydroxyapatite crystals. Calc. Tiss. Res. 1:243-245.
- Ibsen, K. H., R. L. Saunders, and M. R. Urist. 1963. Fluorometric determination of oxytetracycline in biological material. Analytical Biochemistry 5:505-514.
- Jefferts, K. B., P. K. Bergman, and H. F. Fiscus. 1963. Coded wire identification system for macro-organisms. Nature (London) 198:460-462.
- Kleinbaum, D. G. and L. L. Kupper. 1978. Applied regression analysis and other multivariable methods. Duxbury Press. Boston, Mass. 556 p.

- Kobayashi, S., R. Yuki, T. Furui and T. Kosugiyama. 1964. Calcification in fish and shell-fish I. Tetracycline labeling patterns on scales, centrum and otoliths in young goldfish. Bull. Jap. Soc. Sci. Fish. 30:6-13.
- Koenings, J. P., J. Lipton and P. McKay. 1983. The fluorometric determination of the uptake and retention of the antibiotic oxytetracycline in sockeye salmon Oncorhynchus nerka fry: a quantitative approach to tetracycline marking. FRED Report Number 19. ADF&G FRED Division, Juneau, AK. 22 p.
- Koenings, J. P. and J. Lipton. 1983. A fluorometric method for the quantitative determination of submicrogram amounts of oxytetracycline in sockeye salmon Oncorhynchus nerka fry. FRED Report Number 20. ADF&G FRED Division, Juneau, AK. 25 p.
- Kohn, K. W. 1961. Determination of tetracycline extraction of fluorescent complexes. Analytical Chemistry. 33: 862-866.
- Margolis, L. 1963. Parasites as indicators of the geographical origin of sockeye salmon, Oncorhynchus nerka (Walbaum), occurring in the North Pacific Ocean and adjacent seas. Bull. 11, Int. North. Pac. Fish. Comm. 101-156.
- Mugiya, Y., N. Watabe, J. Yamada, J. M. Bean, D. G. Dunkelberger, and M. Shimuzu. 1981. Diurnal rhythm in otolith formation in the goldfish, Carassius auratus. Comp. Biochem. Physiol. 68A:659-662.
- Neilson, J. D., G. H. Green and B. Chan. 1985. Variability in dimensions of salmon otolith nuclei: implications for stock identification and microstructure in interpretation. Fishery Bulletin. 83:81-89.
- Odense, P. H. and V. H. Logan. 1974. Marking atlantic salmon Salmo salar with oxytetracycline. Jour. Fish. Res. Bd. Canada. 31:348-350.
- Schnick, R. A. and F. P. Meyer. 1979. Announcement of compounds registered for fishery users. Prog. Fish. Cult. 41:36-38.
- Scidmore, W. J. and D. E. Oslen. 1969. Marking walleye fingerlings with oxytetracycline antibiotic. Prog. Fish. Cult. Oct.:213-216.

- Stekoll, M. S., W. W. Smoker, and M. M. Rowcroft. 1986. Extraction and analysis of oxytetracycline tags in salmonids. SFS UAJ-8608.
- Sydor, W. J., W. R. Miller III, J. Cairns Jr., and D. Gruber. 1982. Use of box and line plots to assess fish ventilatory behavior in biological monitoring systems. Can. J. Fish. Aquat. Sci. 39:1719-1722.
- Tsukamoto, K. 1985. Mass-marking of ayu eggs and larvae by tetracycline-tagging of otoliths. Bull. Jap. Soc. Sci. Fish. 51:903-911.
- Velleman, P. F., and D. C. Hoaglin. 1983. Applications, basics, and computing of exploratory data analysis. Buxbury Press, Boston, Mass. 354 p.
- Weber D. and G. J. Ridgway. 1967. Marking pacific salmon with tetracycline antibiotics. Jour. Fish. Res. Bd. Canada. 24:849-865.
- Weber D. 1962. The deposition of tetracycline drugs on bones and scales of fish and its possible use for marking. Prog. Fish. Cult. Oct.:150-155.
- Windholz, M., S. Budavari, R. F. Blumetti, and E. S. Otterbein. The Merck Index and encyclopedia of chemicals, drugs and biologicals. 1983. Merck & Co., Inc. Rahway, New Jersey. 1463 p.